

AMENDMENTS TO THE CLAIMS

The listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Withdrawn) A method for characterising an analyte by matrix assisted laser desorption ionization (MALDI) mass spectrometry, which method comprises:
 - (a) labelling the analyte with a light-absorbing label that absorbs light at a pre-determined frequency, to form a labelled analyte;
 - (b) embedding the labelled analyte in a matrix wherein the matrix comprises at least one light-absorbing compound, to form an embedded labelled analyte;
 - (c) desorbing the embedded labelled analyte by exposing it to light having the pre-determined frequency, to form a desorbed analyte; and
 - (d) detecting the desorbed analyte by mass spectrometry, to characterise the analyte.
2. (Withdrawn) The method according to claim 1, wherein the desorbed analyte is directly detected by mass spectrometry.
3. (Withdrawn) The method according to claim 1, in which the desorbed analyte is indirectly detected by mass spectrometry, wherein the analyte further comprises a mass label relatable to the analyte, which method comprises cleaving the mass label from the desorbed analyte and detecting the mass label by mass spectrometry to characterise the analyte.

4. (Withdrawn) The method according to claim 1, wherein the light to which the embedded labelled analyte is exposed is laser light.
5. (Withdrawn) The method according to claim 1, wherein the light-absorbing compound absorbs light at the pre-determined frequency.
6. (Withdrawn) The method according to claim 1, wherein the matrix and light-absorbing label are formed from the same compound.
7. (Withdrawn) The method according to claim 1, wherein the matrix is a solid matrix or liquid matrix.
8. (Withdrawn) The method according to claim 7, wherein the matrix is a liquid matrix comprising nitrobenzyl alcohol.
9. (Withdrawn) The method according to claim 1, wherein the matrix comprises an acid matrix or a basic matrix.
10. (Withdrawn) The method according to claim 1, wherein the matrix comprises 3-hydroxypicolinic acid, 2,5-dihydroxybenzoic acid, or 4-hydroxy-alpha-cyanocinnamic acid.
11. (Withdrawn) The method according to claim 1, wherein the light-absorbing label is formed from a dye.
12. (Withdrawn) The method according to claim 11, wherein the dye is a non-fluorescent dye.

13. (Withdrawn) The method according to claim 12, wherein the dye comprises 4-dimethylaminoazobenzene-4'-sulphonyl chloride (dabsyl chloride), 3-hydroxypicolinic acid, 2,5-dihydroxybenzoic acid, or 4-hydroxy- α -cyanocinnamic acid.

14. (Withdrawn) The method according to claim 1, wherein the analyte comprises a protein, a polypeptide, a peptide, a peptide fragment, or an amino acid.

15. (Previously Presented) A method for characterising a polypeptide, which method comprises the steps of:

- (a) cleaving the polypeptide with a sequence specific cleavage reagent to form peptide fragments;
- (b) capping one or more ϵ -amino groups that are present with a lysine reactive agent;
- (c) analyzing the peptide fragments according to the method of claim 1 to form a mass fingerprint for the polypeptide; and
- (d) determining the identity of the polypeptide from the mass fingerprint.

16. (Previously Presented) A method for characterising a population of polypeptides, which method comprises the steps of:

- (a) separating one or more polypeptides from the population;
- (b) cleaving one or more polypeptides with a sequence specific cleavage reagent to form peptide fragments;
- (c) capping one or more ϵ -amino groups that are present with a lysine reactive agent;

(d) analysing the peptide fragments according to the method of claim 1 to form a mass fingerprint for one or more polypeptides; and

(e) determining the identity of one or more polypeptides from the mass fingerprint.

17. (Previously Presented) A method for comparing a plurality of samples, each sample comprising one or more polypeptides, which method comprises the steps of:

(a) separating one or more polypeptides from each of the samples;

(b) cleaving the polypeptides with a sequence specific cleavage reagent to form peptide fragments;

(c) capping one or more ϵ -amino groups that are present with a lysine reactive agent;

(d) analysing peptide fragments according to the method of claim 1 to form a mass fingerprint for one or more polypeptides from the samples; and

(e) determining the identity of one or more polypeptides in the samples from one or more mass fingerprints.

18. (Previously Presented) The method according to claim 15, wherein the lysine-reactive agent is a labelled lysine-reactive agent.

19. (Previously Presented) A method for comparing a plurality of samples, each sample comprising one or more polypeptides, which method comprises:

(a) capping one or more ϵ -amino groups that are present in each sample with a labelled lysine reactive agent;

(b) pooling the samples;

- (c) separating one or more polypeptides from the pooled samples;
 - (d) cleaving the polypeptides with a sequence specific cleavage reagent to form peptide fragments;
 - (e) analyzing peptide fragments according to the method of claim 1 to form a mass fingerprint for one or more polypeptides from the samples; and
 - (f) determining the identity of one or more polypeptides in the samples from one or more mass fingerprints;
- wherein the same label is employed for polypeptides or peptides from the same sample, and different labels are employed for polypeptides or peptides from different samples, such that the sample from which a polypeptide or peptide originates can be determined from its label.

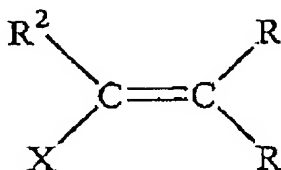
20. (Previously Presented) The method according to claim 19, wherein the sequence specific cleavage agent cleaves the one or more polypeptides on the C-terminal side of a lysine residue.

21. (Withdrawn) The method according to claim 19, wherein the specific cleavage agent comprises Lys-C or Trypsin.

22. (Withdrawn) The method according to claim 19, wherein the peptide fragments having capped ϵ -amino groups are removed by affinity capture, and wherein the lysine reactive agent comprises biotin.

23. (Previously Presented) The method according to claim 19, wherein the lysine reactive agent comprises a hindered Michael reagent.

24. (Previously Presented) The method according to claim 23, wherein the hindered Michael agent comprises a compound having the following structure:



wherein X is an electron withdrawing group that is capable of stabilizing a negative charge; the R groups independently comprise a hydrogen, a halogen, an alkyl, an aryl, or an aromatic group with the proviso that at least one of the R groups comprises a sterically hindering group; and the group R² comprises a hydrogen, a halogen, a hydrocarbon group, an electron withdrawing group, or a linker capable of attachment to an affinity capture functionality or a solid phase support.

25. (Withdrawn) A labelled analyte compound, which compound has the following structure:



wherein D comprises a light absorbing label, M comprises a mass modifier, L comprises a linker and A comprises an analyte.

26. (Withdrawn) The compound of claim 25, wherein D comprises a non-fluorescent dye.

27. (Withdrawn) The compound of claim 26, wherein the non-fluorescent dye comprises a cinnamic acid derivative, a nicotinic acid derivative, a picolinic acid derivative, a

hydroxybenzoic acid derivative, a methoxybenzoic acid derivatives, or a sinapinic acid derivative.

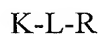
28. (Withdrawn) The compound of claim 26, wherein the non-fluorescent dye comprises 4-dimethylaminoazobenzene-4'-sulphonyl chloride (DABSYL chloride), 3-hydroxypicolinic acid, 2,5-dihydroxybenzoic acid, or 4-hydroxy- α -cyanocinnamic acid.

29. (Withdrawn) The compound of claim 25, wherein M is a compound formed from an aryl ether or an oligomer formed from 2 or more aryl ether units.

30. (Withdrawn) The compound of claim 25, wherein the linker comprises $-\text{CR}_2-\text{CH}_2-\text{SO}_2-$, $-\text{N}(\text{CR}_2-\text{CH}_2-\text{SO}_2)_2$, $-\text{NH}-\text{CR}_2-\text{CH}_2-\text{SO}_2-$, $-\text{CO}-\text{NH}-$, $-\text{CO}-\text{O}-$, $-\text{NH}-\text{CO}-\text{NH}-$, $-\text{NH}-\text{CS}-\text{NH}-$, $-\text{CH}_2-\text{NH}-$, $-\text{SO}_2-\text{NH}-$, $-\text{NH}-\text{CH}_2-\text{CH}_2-$, or $-\text{OP}(=\text{O})(\text{O})\text{O}-$.

31. (Withdrawn) The compound of claim 25, wherein A comprises a protein, a polypeptide, a peptide, a peptide fragment, or an amino acid.

32. (Withdrawn) A compound for labelling an analyte, which compound has the following structure:



wherein K is a mass marker comprising a cinnamic acid derivative, a nicotinic acid derivative, a picolinic acid derivative, a hydroxybenzoic acid derivative, a methoxybenzoic acid derivative or a sinapinic acid derivative; L comprises a linker; and R comprises a reactive functionality for attaching the compound to an analyte.

33. (Withdrawn) The compound of claim 32, wherein K comprises a non-fluorescent dye compound.

34. (Withdrawn) The compound of claim 32, wherein K further comprises a compound formed from an aryl ether or an oligomer formed from 2 or more aryl ether units.

35. (Withdrawn) The compound of claim 32, wherein the linker comprises -CR₂-CH₂-SO₂-, -N(CR₂-CH₂-SO₂-)₂-, -NH-CH₂-CH₂-SO₂-, -CO-NH-, -CO-O-, -NH-CO-NH-, -NH-CS-NH-, -CH₂-NH-, -SO₂-NH-, -NH-CH₂-CH₂-, -OP(=O)(O)O- and , or an amide linkage through the acid groups of M.

36. (Withdrawn) The compound of claim 32, wherein the analyte is a protein, polypeptide, peptide, peptide fragment, or amino acid.

37. (Withdrawn) The compound of claim 32, wherein R comprises an ester group, an acid anhydride group, an acid halide group an N-hydroxysuccinamide group, a pentafluorophenyl ester group, a maleimide group, an alkenyl sulphone group, or an iodoacetamide group.

38. (Withdrawn) The 4 compound of claim 25, further comprising an affinity ligand.

39. (Withdrawn) The compound of claim 38, wherein the affinity ligand comprises biotin.

40. (Withdrawn) The compound of claim 25, further comprising an ionizable moiety.

41. (Withdrawn) The compound of claim 40, wherein the ionizable moiety is selected from the group consisting of a tertiary amino group, guanidino groups and sulphonic acid group.

42. (Withdrawn) The compound of claim 25, comprising a cinnamic acid functionality.

43. (Withdrawn) An array for labelling an analyte, which array comprises two or more compounds of claim 32, wherein each compound has a different mass.

44. (Withdrawn) The array of claim 43, wherein the difference in mass of each compound is achieved by isotopic substitution.

45. (Withdrawn) A kit for characterising an analyte by matrix assisted laser desorption ionization (MALDI) mass spectrometry, which kit comprises:

(a) one or more light absorbing labels having a reactive functionality for attaching the label to an analyte; and

(b) a compound for forming a matrix, which compound absorbs light at the same frequency as the light-absorbing label.

46. (Withdrawn) The kit of claim 45, wherein the light absorbing label comprises a compound, having the following structure:



wherein K is a mass marker comprising a cinnamic acid derivative, a nicotinic acid derivative, a picolinic acid derivative, a hydroxybenzoic acid derivative, a methoxybenzoic acid derivative, or a sinapinic acid derivative; L comprises a linker; and R comprises a reactive functionality for attaching the compound to an analyte.

47. (Withdrawn) A kit for characterising an analyte by matrix assisted laser desorption ionization (MALDI) mass spectrometry, which kit comprises:

- (a) the compound of claim 32; and
- (b) an ion exchange resin.

48. (Withdrawn) The kit of claim 47, wherein the compound comprises an ionizable moiety that forms a positive charge, and wherein the ion exchange resin comprises a cation exchange.

49. (Withdrawn) The kit of claim 47, wherein the compound comprises an ionizable moiety that forms a negative charge, and wherein the ion exchange resin comprises an anion exchange.

50. (Withdrawn) The method according to claim 3, wherein the mass label is the light-absorbing label.

51. (Previously Presented) The method according to claim 15, further comprising reducing cysteine disulphide bridges in the polypeptide to form free thiols and capping the free thiols, prior to cleaving the polypeptide.

52. (Previously Presented) The method according to claim 15, further comprising deactivating the cleavage agent after cleaving the polypeptide.

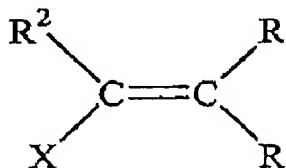
53. (Previously Presented) The method according to claim 15, wherein the sequence specific cleavage agent cleaves each isolated polypeptide on the C-terminal side of a lysine residue.

54. (Withdrawn) The method according to claim 15, wherein the sequence specific cleavage agent comprises Lys-C or Trypsin.

55. (Previously Presented) The method according to claim 15, wherein the peptide fragments having capped ϵ -amino groups are removed by affinity capture, and wherein the lysine reactive agent comprises biotin.

56. (Previously Presented) The method according to claim 15, wherein the lysine reactive agent comprises a hindered Michael reagent.

57. (Previously Presented) The method according to claim 56, wherein the hindered Michael agent comprises a compound having the following structure:



wherein X is an electron withdrawing group that is capable of stabilizing a negative charge; the r groups independently comprise a hydrogen, a halogen, an alkyl, an aryl, or an aromatic

group with the proviso that at least one of the R groups comprises a sterically hindering group; and the group R^2 comprises a hydrogen, a halogen, a hydrocarbon group, an electron withdrawing group, or a linker capable of attachment to an affinity capture functionality or a solid phase support.

58. (Previously Presented) The method according to claim 16, further comprising reducing cysteine disulphide bridges in the polypeptide to form free thiols and capping the free thiols, prior to cleaving the polypeptide.

59. (Previously Presented) The method according to claim 16, further comprising deactivating the cleavage agent after cleaving the polypeptide.

60. (Previously Presented) The method according to claim 17, further comprising reducing cysteine disulphide bridges in the polypeptide to form free thiols and capping the free thiols, prior to cleaving the polypeptide.

61. (Previously Presented) The method according to claim 17, further comprising deactivating the cleavage agent after cleaving the polypeptide.

62. (Previously Presented) The method according to claim 19, further comprising reducing cysteine disulphide bridges in the polypeptide to form free thiols and capping the free thiols, prior to capping one or more ϵ -amino groups.

63. (Previously Presented) The method according to claim 19, further comprising deactivating the cleavage agent after cleaving the polypeptides.

64. (Withdrawn) The compound of claim 25, further comprising an affinity ligand.
65. (Withdrawn) The compound of claim 64, wherein the affinity ligand comprises biotin.
66. (Withdrawn) The compound of claim 25, further comprising an ionizable moiety.
67. (Withdrawn) The compound of claim 66, wherein the ionizable moiety is selected from the group consisting of a tertiary amino group, guanidino group, and sulphonic acid group.
68. (Withdrawn) The compound of claim 25, further comprising a cinnamic acid functionality.
69. (Withdrawn) The compound of claim 33, wherein the the non-fluorescent dye comprises 4- dimethylaminoazobenzine-4'-sulphonyl chloride (DABSYL chloride), 3-hydroxypicolinic acid, 2,5- dihydroxybenzoic acid, or 4-hydroxy-alpha-cyanocinnamic acid.
70. (Withdrawn) The compound of claim 37, wherein the acid halide group is acid chloride.